metric to quantify how much information was reflected in the paths. For wild-type bacteria the information in the trajectories is essentially constant across all tumble angles (and several gradients). However, if either the angle variance or the rotational diffusion is set to zero, distinct minima appear in the trajectory information appear at 0, 90, and 180 degrees - and there are broad maxima around 70 and 110 degrees. In simulations where both the angle variance and the rotational diffusion are set to zero, the trajectory information exhibits several more prominent minima, notably at 135, 60, and 45 degrees. We suggest that these minima arise because angles that are small integer fractions of 180 or 540 degrees increase the likelihood of backtracking - thus reducing the new space explored by the bacterium. When a bacterium does tumble, it should do so in a way as to explore as much new space as possible in order to optimize information gathering. Notably that is not 90 degrees, but one maximum is close to the normal tumble angle of 68 degrees for E. coli.

3035-Pos Board B465

Antibodies Change the Mechanics of Adhesion Fimbriae - a Case Study of CS20 Fimbriae Expressed by Enterotoxigenic Escherichia Coli

Narges Mortezaei[1], Bhupender Singh[1], Bernt Eric Uhlin[2], Stephen J. Savarino[3], Esther Bullitt[4], Magnus Andersson[1].
[1]Department of Physics, Umeå University, Umeå, Sweden, [2]The Laboratory for Molecular Infection Medicine Sweden (MIMS) and Department of Molecular Biology, Umeå University, Umeå, Sweden, [3]Enteric Diseases Department, Infectious Diseases Directorate, Naval Medical Research Center, Silver Spring, MD, USA, [4]Department of Physiology and Biophysics, Boston University School of Medicine, Boston, MA, USA.

Enterotoxigenic Escherichia coli (ETEC) express a variety of fimbriae that mediate adhesion to host epithelial cells. It has been shown that the ability of a fimbriated bacterial cell to attach and stay attached to host cells does not merely depend on the adhesin expressed distal of the fimbriae but also the biomechanical properties of the fimbriae are vital for sustained adhesion. Fimbriae can significantly extend under a constant force when exposed to an external force and therefore reduce the load on the adhesin, which is believed to help bacteria to withstand external forces applied by various body defense systems. Thus, it is thought that the fimbrial shaft and adhesin have co-evolved for optimal function when bacteria attach to host cells. To investigate if antibodies, normally found in the intestines, affects the biomechanical properties of fimbriae, we exposed CS20 fimbriae expressed by ETEC to anti-fimbrial antibodies and measured these properties using optical tweezers force spectroscopy. Our data show a change in the force required to extend the fimbriae and that the elasticity is significantly reduced by the presence of antibodies. The reduced elasticity, likely due to cross-linking of fimbrial subunits, could thus be another assignment for antibodies; in addition to their mission in marking bacteria as foreign, our data indicate that antibodies physically compromise fimbrial function. To further confirm interaction of antibodies to their specific target we performed western blot analysis, transmission electron microscopy and immunofluoresence microscopy. In the presence of antibodies, we suggest that our assay and results will be a starting point for further studies aimed at inhibiting bacterial adhesion by antibodies.

3036-Pos Board B466

Single Cell Dynamics Drive Turbulent Flow in the Collective Motion of Bacteria

Alex Hamby, Charles Wolgemuth.
University of Arizona, Tucson, AZ, USA.

In most areas of biology, the principal confounding factor is the complexity. Biology from the cellular level to the ecosystem involves the action of a multitude of individuals that come together and achieve specific tasks. For example, at the single cell level, the binding and unbinding of cytoskeletal proteins conspire to allow a cell to move across surfaces or through the extracellular matrix. At the tissue level, these motile cells act together to heal wounds or form cancer metastases. Tissues come together to form organisms, which form societies, and so on. Here we use dense communities of swimming bacteria to understand how collective behavior arises out of the actions of an individual. At high density, rod-shaped bacteria produce complex fluid flows that include vortices and jets. These flows arise partially due to the dipole forces that each bacterium exerts on the surrounding fluid. By confining *Bacillus subtilis* or *Escherichia coli* within Hele-Shaw cells of controllable depth, we probe how individual biophysical parameters, such as shape, speed, external drag, and chemotaxis, affect the resulting collective behavior in this system. We then compare our results to predictions from a two-phase fluid model that is based on the single-cell physics of a swimming bacterium. Comparison of our experimental and simulation results show that the collective behavior in this system is largely determined by the biophysics of the single organism. The physics of these dense communities has many similarities to actomyosin systems, as well as to collective systems of epithelial cells. Therefore, our results are likely broadly applicable to a wide range of problems in cell migration, from the single cell to the collective.

3037-Pos Board B467

Coupling Scheme of the Rotary Motor Thermophilic F1

Kengo Adachi[1], Kazuhiro Oiwa[2], Masasuke Yoshida[3], Kazuhiko Kinosita Jr[1].
[1]Dept. of Physics, Waseda Univ., Tokyo, Japan, [2]Adv. ICT Res. Inst., NICT, Kobe, Japan, [3]Dept. of Mol. Biosci., Kyoto Sangyo Univ., Kyoto, Japan.

Thermophilic F_1 (TF_1) is an ATP-driven rotary molecular motor driven by sequential hydrolysis of ATP in three catalytic sites. Rotation occurs in steps of 120° per ATP, and the 120° step is further resolved into 80-90° and 40-30° substeps. In the standard coupling scheme, ATP binding starts rotation from an ATP-waiting angle at 0°, and at ~200° the ATP is cleaved into ADP and Pi, and the ADP is released around 240° after a third ATP is bound. Pi release is at 200° or 320°, yet unsettled. With human mitochondrial F_1 (HF_1), Suzuki (2014) has indicated different angle dependence: cleavage occurs at 210° and Pi release at 305°. A peculiar finding in HF_1 was that supposedly slowly hydrolyzed ATPγS not only lengthened the dwell at 210° but to much greater extent the 305° dwell, implying that the thio-Pi release is much slower than the ATPγS cleavage. We thus re-examined under a microscope how ATPγS affects TF_1. With fluorescently (Cy3) labeled ATPγS we observed a remarkably long dwell at only 200° after its binding. With unlabeled ATPγS mixed in ATP, we observed only one long dwell per ATPγS binding, compared to two consecutive long dwells in HF_1. The long dwell with unlabeled ATPγS, presumably at 200°, comprised two reactions with rates 460 s^{-1} and 30 s^{-1}, compared to 2400 s^{-1} and 820 s^{-1} with regular ATP. We have yet to decide which corresponds to cleavage and (thio-)Pi release, but kinetic difference from HF_1 is obvious: either (thio-)Pi release occurs at 200° in TF_1, or thio-Pi release is not slow in TF_1. Nonhydrolyzable Cy3-AMPPNP halted rotation at 200° after binding, implying that ATP cleavage occurs at 200° or before, a conclusion previously drawn on the assumption of slow cleavage of ATPγS and a mutant.

Energy Transduction, Electron and Proton Transfer, and Light Harvesting

3038-Pos Board B468

Exploring the Staphylococcus Epidermidis Respiratory Chain

Cristina Uribe Alvarez[1,2], Natalia Chiquete-Félix[1,3], Salvador Uribe-Carvajal[1], Antonio Peña[1].
[1]Inst FIsiol Celular, UNAM, Mexico, Mexico, [2]Molec Genetics, INS FISIOL CELULAR, UNAM, Mexico City, Mexico, [3]Molec genetics, INST FISIOL CELULAR, UNAM, Mexico city, Mexico.

Staphylcoccus epidermidis does not invade healthy tissues. However, it has been identified as a major cause of nosocomial infections because of its ability to infect polymer surfaces such as catheters or intra-cardiac valves, forming biofilms and evoking a reaction from the host that eventually leads to removal of prosthesis. S. epidermidis is responsible for 50-70% of catheter-related infections and 30 to 43% of perioperative implant infections. S. epidermidis can survive in a wide range of environments and oxygen concentrations ([O2]). It may grow in atmospheric oxygen levels, in micro-aerobic environments, including normal tissues with [O2]= 3 to 5% and even in pathologically altered tissues where O2 tensions may reach zero. S. epidermidis increases its propensity to form biofilms as [O2] decreases. Bacteria may contain different terminal oxidases that allow them to cope with different [O2]. Also, respiratory chain-branching aids the cell to survive in the presence of toxic substances. Understanding the plasticity of the respiratory chain is necessary to understand the physiology and pathogenicity of S. epidermidis. In this species, we found two terminal oxidases expressed during growth in different [O2]: bo is always present, while aa3 is expressed in aerobic conditions but not in microaerophilic conditions nor in KCN plus a non-fermentable carbon source. In Oxygen consumption experiments, the preference for different substrates varied depending on [O2] exposure during growth.

3039-Pos Board B469

New Perspectives on Quinol Binding Motifs at the bc1 Complex Based on MD Simulations

Angela M. Barragan[1,2], Abhishek Singharoy[2], Anthony R. Crofts[3,4], Klaus Schulten[1,2], Ilia A. Solov'yov[5].
[1]Physics, University of Illinois at Urbana-Champaign, Urbana, IL, USA, [2]Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana–Champaign, Urbana, IL, USA, [3]Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL, USA, [4]Center for Biophysics and Computational Biology, University of Illinois at Urbana–Champaign, Urbana, IL, USA, [5]Physics, University of Southern Denmark, Odense, Denmark.

The bc1 complex is a central player in the conversion of energy into ATP synthesis in photosynthesis and respiration, and its overall mechanism, the Q-cycle, is well known. However, the quinol-protein interaction that initiates Q-cycle at the Qo-binding site have not yet been described. Employing classical MD

simulations in tandem with DFT calculations, the quinol binding motifs to the Qo-site of bc1 complex is investigated for a range of Qo-site protonation states. The computations revealed a novel configuration of the key side groups at the Qo-site site, such as H156, Y147 and E295, that stabilize the reaction complex and provide an optimal configuration prior to the charge transfer reactions between quinol and iron-sulfur cluster of the Rieske protein. Re-arrangements in the E295 and Y147 side chains were observed in all our simulations, showing intermediate bridging hydrogen bonding between quinol and E295, not observed before. Simulations were extended to study cytochrome c2 docking to cytochrome c1, required for the removal of electrons from the bc1 complex. We found, the docking interface is characterized by a semicircular arrangement of electrostatic residues that draws the cytochrome c2 and bc1 complex into an encounter complex, a hydrophobic minimal core to facilitate electron transfer, and mobility mismatch between the bound surfaces to induce post-electron transfer undocking.

3040-Pos Board B470

Glutathione S-Transferase Kappa 1 Knockdown Exacerbates Complex-III-Mediated ROS Production in H9c2 Cardiac Cells

Kyriakos N. Papanicolaou, Agnieszka Sidor, Jasma Rucker,
Brian O'Rourke, D. Brian Foster.
Cardiology, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Background: Mitochondria produce reactive oxygen species (ROS) that are scavenged by local antioxidant enzymes. Glutathione (GSH) is key intermediate in many of these reactions and its availability determines the antioxidant capacity of mitochondria. Glutathione S-transferases (GSTs) are known to consume GSH during xenobiotic detoxification but their involvement in ROS scavenging is less clear. Gstκ1 was originally identified as a novel mitochondrial GST but its role there remains unknown.

Objective: To examine whether loss of Gstκ1 affects the [GSSG/GSH] ratios in cytosol and mitochondria of the cardiac-derived cell line H9c2.

Methods: Knockdown of Gstκ1 (Gstκ1-KD) in H9c2 cells was achieved by transfection with siRNA. Changes in [GSSG/GSH] were monitored using genetically-encoded ratiometric sensors that localize to cytosol or mitochondria. Global oxidative stress was induced with hydrogen peroxide (HP). Mitochondrially-targeted oxidative stress was induced by the superoxide generator di-methoxy-naphtho-quinone (DMNQ) and by antimycin-A (AA) which interacts specifically with mitochondrial complex III.

Results: Treatment with HP elevatedcytosolic [GSSG/GSH] ratios equally in both control and Gstκ1-KD cells. Mitochondrial oxidative stress elicited by HP or DMNQ likewise increased mitochondrial [GSSG/GSH] and was unaffected by Gstκ1-KD. Inhibition of Complex III by AA decreased mitochondrial [GSSG/GSH] persistently in control cells. However, in Gstκ1-KD cells exposed to AA, the decrease was transient and was followed by a sustained increase in [GSSG/GSH].

Conclusions: Here we examine for the first time the role of Gstκ1 in ROS scavenging in cardiac cells. While our data suggest that Gstκ1 does not participate in the general response against exogenous ROS, they indicate that low levels of this enzyme associate with increased superoxide production from mitochondrial complex III. Our findings may provide insights for experimental models of cardiac disease where Gstκ1 expression is downregulated.

3041-Pos Board B471

Evaluation of Heme Peripheral Groups Interactions in Low-Dielectric Constant Media

Jose F. Cerda, **Alaina T. Stockhausen**, Nicolette D. Wilkes,
Kathleen R. Silva, Allyson R. Langley, Mary C. Malloy,
Brady O. Werkheiser.
Chemistry, Saint Joseph's University, Philadelphia, PA, USA.

In this study, we measured the contributions of the ionization of the heme propionates to the reduction potentials of heme b and heme a (bis)N-methylimidazole complexes in various low-dielectric constant conditions. Additionally, we measured the effects of H-bond to the heme a formyl group on the reduction potential of the heme. The performed electrochemical measurements show that ionization of the heme propionates lead to the largest redox change in dichloromethane with no electrolyte. The measured reduction potential changes for heme b and heme a were - 55 and −47 mV (±10 mV) per ionized propionate, respectively. For heme a, the study demonstrates how the dielectric constant of the medium is important in the magnification of the αpKa upon redox-linked ionization of the heme propionates and their roles in the proton pump of cytochrome c oxidase. Additionally, we carried out a detailed study on the H-bonding properties of heme a model compounds (copper mono- and di-acetyl porphyrins) and the effects of the dielectric constant of the solvent on the measured dissociation constant (Kd) between various H-bond donors and the copper porphyrins. Our measurements show that H-bonds between the copper porphyrins and TFA or phenol are significant in benzene and dichloromethane, but extremely weak in the presence of acetonitrile. This study shows how the dielectric constant of the medium also plays a role in modulating the properties of heme a through H-bonding of its formyl group.

3042-Pos Board B472

Theoretical Investigation of the Primary Event in Proteorhodopsin Activation

Choongkeun Lee, Blake Mertz.
Chemistry, West Virginia University, Morgantown, WV, USA.

Retinal proteins are α-helical transmembrane proteins that have the potential for applications such as memory storage (bacteriorhodopsin) and optogenetics (channelrhodopsin). Proteorhodopsin (PR) is a recently discovered microbial retinal protein which acts as a proton pump, similar to bacteriorhodopsin. Initial activation of PR occurs when the covalently bound chromophore, retinal, absorbs a photon and undergoes an all-*trans* → 13-*cis* isomerization. Despite several similarities between bacteriorhodopsin and PR, the details of the activation process remain unclear. We investigated the photoisomerization of retinal in PR using a hybrid quantum mechanical (QM) and molecular mechanics (MM) approach. Two photoexcited states were identified. Both retinal structures have a 13-*cis*, 15-*anti* conformation. However, one does not involve a hydrogen bond between the Schiff base and a water molecule, while the other structure does, dependent on the rotational direction of the isomerization. The former is more stable than the latter by about 0.9 eV and the former also has a lower energy barrier of the photoisomerization by about 0.2 eV than the latter. Upon photoisomerization, PR reaches the former excited state, subsequently proceeding through a slow rearrangement to form the hydrogen bond between the Schiff base and water and generate the latter excited state. We believe the former structure to be indicative of the K state, with the latter structure more characteristic of an L-like state. This L-like state is spectroscopically silent and most likely extremely transient, due to the low energy barrier (0.1 eV) of the proton transfer from the Schiff base to the proton acceptor, D97. A detailed understanding of the initial events in the activation process is essential to utilizing PR as a light-driven component in potential technological applications.

3043-Pos Board B473

The Electron Transfer in Ferredoxins

Kelly N. Tran, Toshiko Ichiye.
Chemistry, Georgetown University, Washington, DC, USA.

The 2[4Fe-4S]-ferredoxins are ubiquitous water-soluble electron shuttles found in all bacteria. The intramolecular "self-transfer" electron transfer between the two [4Fe-4S] clusters is an ideal model system for studying electron transfer independent of association of the donor and acceptor and may be considered as the limit of a very tightly bound complex. Thus, the effects of environmental factors on electron transfer rates can be focused on. *Clostridium acidurici* ferredoxin (CaFd) is a small (5.9 kDa, 55 residues), pseudo-symmetric protein containing two $[Fe_4S_4(SR)_4]^{2-}$ redox sites which are separated by ~12 Å, the typical distance of biological electron transfer. Molecular dynamics simulations of CaFd are performed using different force fields to test their performance. The environmental reorganization energy λ is calculated from the simulations and compared to experiment.

3044-Pos Board B474

Internal Switches Modulating Electron Flow in bc1 Complex

Muhammad Hagras, Alexei A. Stuchebrukhov.
Biophysics, University of California, Davis, Davis, CA, USA.

Ubiquinol-cytochrome c oxidoreducase (BC1) protein complex is a smart machine which employs an internal signal transduction network, regulated by the binding of natural ligands to either Qo or Qi sites, to modulate electron transfer rates between different redox pairs. Binding of natural ligands or some inhibitors leads to local conformational changes which propagate through protein and control the conformation of key residues involved in the electron tunneling pathway. Aromatic-aromatic interactions are highly utilized in this internal network since the key residues are aromatic in nature. Molecular dynamics simulations of native BC1, natural ligand and inhibitor-bound BC1 homo-dimers in membrane were performed to investigate and compare the dynamics of those key residues in their respective environments. In addition, molecular dynamics simulations reveals the transduction pathway induced by the binding of a ligand or inhibitor at their sites. Electron tunneling calculations show that there is a substantial correlated change of the electron transfer rates between different redox pairs depending on the binding of natural ligands or inhibitors.

3045-Pos Board B475

Energetics of Lateral Membrane Proton Diffusion

Ewald Weichselbaum, Denis Knyazev, Peter Pohl.
Molecular and Membrane Biophysics, Institute of Biophysics, Linz, Austria.

The diffusion of protons along biological membranes is vitally important for cellular energetics. The weak dependence of both migration speed and span on lipid composition suggests that proton migrate along water (Springer et al., 2011). However, it is unclear how to reconcile the apparently high proton affinity to the phase boundary (Zhang et al., 2012) with the poor proton acceptability of water, i.e. with the low pK(=0) value of water. Here we monitored the